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(54) Title: SYNTHESIS OF N-ACETYL NEURAMINIC ACID DERIVATIVES

(57) Abstract

A method for the preparation of 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-eno-pyranosonic acid by reaction of 5-acetamido-2,3,4,5-tetradeoxy-4-amino-D-glycero-D-galacto-non-2-eno-pyranosonic acid with pyrazole-1H-carboxamidine or a derivative thereof in aqueous medium.

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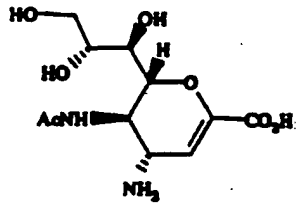
SYNTHESIS OF N-ACETYL NEURAMINIC ACID DERIVATIVES

The present invention relates to a process for the preparation of derivatives of N-acetyl neuraminic acid. More particularly the invention relates to a process for the preparation of 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galactonon-2-enopyranosonic acid (the 4-guanidino analogue of DANA; also known as 5-(acetylamino)-2,6 anhydro-3,4,5-trideoxy-4-guanidino-D-glycero-D-galacto-non-2-enonic acid) and derivatives thereof.

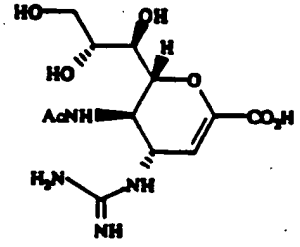
PCT/AU91/00161 (publication no. WO91/16320) describes a number of derivatives of 5-acetamido-2,3,5-trideoxy-D-glycero-D-galacto-non-2-enopyranosonic acid (2,3-dideoxy-2,3-didehydro-N-acetyl-neuraminic acid; DANA) including the 4-guanidino analogue of DANA. The 4-guanidino analogue of DANA is prepared by the reaction of the corresponding O-acyl protected 4-amino analogue of DANA by reaction with S-methylisourea followed by deprotection.

We have now found a method of preparing the 4-guanidino analogue of DANA directly from the unprotected 4-amino analogue of DANA.

The structure of the 4-amino and 4-guanidino analogues of DANA are shown below:

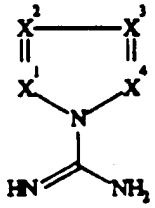


4-amino analogue of DANA



4-guanidino analogue of DANA

The invention thus provides in a first aspect a method for the preparation of 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-eno-pyranosonic acid which comprises the reaction of 5-acetamido-4-amino-2,3,4,5-tetradeoxy D-glycero-D-galacto-non-2-enopyranosonic acid with a compound of formula (I)



(I)

where at least one of X^1, X^2, X^3 and X^4 is C-R and the remainder are C-R or N and each R is the same or different and is H, C_{1-6} alkyl, unsubstituted or substituted phenyl, unsubstituted or substituted phenyl C_{1-4} alkyl or an electron withdrawing group.

When R in the compound of formula (I) is an electron withdrawing group any such group may be employed. Such groups will be evident to those skilled in the art and include for example carboxyl, nitro and cyano.

The compound of formula (I) may be employed either as the free base or as an acid addition salt. Suitable salts include those derived from inorganic or organic acids such as hydrochloric, hydrobromic, sulphuric, nitric, perchloric, fumaric, maleic, phosphoric, glycollic, lactic, salicylic, succinic, toluene- p-sulphonic, tartaric, acetic, citric, methanesulphonic, formic, benzoic, malonic, naphthalene-2-sulphonic and benzenesulphonic acids.

The preferred compounds of formula (I) are pyrazole-1H-carboxamidine and derivatives thereof bearing a C_{1-6} alkyl group on the pyrazole ring.

A particularly preferred compound of formula (I) is pyrazole-1H-carboxamidine either as the free base or, preferably, as an acid addition salt such as pyrazole-1H-carboxamidine hydrochloride (PCH).

The reaction is conveniently carried out in aqueous medium. By aqueous medium is meant any liquid medium comprising a substantial amount, for

example 50% or more, of water. Preferably the aqueous medium comprises water alone.

The reaction may be carried at ambient or elevated temperature for example 30°C to 70°C. Preferably the reaction is carried out at about 50-55°C.

The molar ratio of the 4-amino analogue of DANA to the compound of formula (I) employed in the reaction may be from about 1:1 to about 1:10 for example 1:1 to 1:3. Preferably the compound of formula (I) is employed in a molar excess of about 1.5 - 2 fold, e.g. about 1.8 fold.

The reaction is carried out at a pH range of about 6 to about 9. The pH range may vary within this range during the reaction.

Where the compound of formula (I) is employed in the form of an acid addition salt the pH may be maintained in the desired range by addition of one or more inorganic or organic bases. Such bases include for example alkali metal hydroxides and carbonates such as lithium hydroxide, sodium carbonate or sodium bicarbonate and amines such as triethylamine, imidazole or 1,8-diazabicylo [5.4.0]undec-7-ene (DBU). The amount of base required to maintain the desired pH will depend upon the particular base(s) employed. Quantities of the base(s) required to control the pH will be apparent to those skilled in the art.

The desired 4-guanidino analogue of DANA may be isolated by any conventional method from the reaction mixture, for example by crystallisation or chromatography. In particular the 4-guanidino analogue of DANA may be isolated by treatment of an aqueous solution with a water miscible organic solvent in which the compound is insoluble. Such solvents include for example aliphatic alcohols such as methanol and *iso*propyl alcohol (IPA) and acetone.

The present invention is further described by the following examples which are for illustrative purposes only and should not be construed as a limitation of the invention.

Example 1

5-Acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid

To a stirred solution of 5-acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonic acid as the trihydrate (58.05g; 169mmol) in water (300ml) at 33°C was added in one lot pyrazole-1H-carboxamidine hydrochloride (600mmol, 87.95g) followed by triethylamine (60ml, pH = 8.4) and the reaction stirred for 5 hrs.

The crude reaction mixture was added to rapidly stirred methanol (900ml). Stirring was continued overnight and the precipitated solid was collected, washed with 4:1 MeOH: H_2O (250ml), air dried and dried in a vacuum oven (42^0C) for 2 h. Yield = 46.7g.

The so obtained product (45.5g) was suspended in water (518ml) and heated with rapid stirring. The so obtained solution was rapidly filtered and allowed to cool to ambient temperature. The solution was further cooled (ice-water bath) to 3^0C. *Iso*propyl alcohol (450ml) was added dropwise to the cold solution over 1hr and stirring continued over 1.5hr. The precipitated solid was filtered off, and dried at 40^0C to give the title compound (30.4g) identical with authentic material.

Example 2

5-Acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-enopyranosonic acid

A mixture of 5-acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonic acid as the trihydrate (15.0g), pyrazole-1H-carboxamidine hydrochloride (7.55g) and imidazole (11.55g) in water (52.5ml) was stirred and heated at 50-55°. After 18h the resulting slurry was treated with acetone (150ml) over 15 min. maintaining the vesel contents at 50-55° . The mixture was then cooled to 15-20° and after a further 3h the product was collected by vacuum filtration. The bed was washed with 4:1 acetone/water (2x25ml) and then

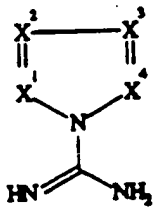
acetone (25ml). The product was air dried at ambient temperature to give the title compound (10.98g).

PMR(D_2O) 2.04 (3H, s), 3.67 (2H, m), 3.93 (2H, m), 4.23 (1H,m), 4.42 (2H,m) 5.63 (1H, d, J 2.5Hz).

IR (Nujol) 3428, 3338, 3253; NH, OH
1692, 1666, 1648, 1619, 1575; CO (CH_3CONH, CO_2^-), CN.

Claims

1. A method for the preparation of 5-acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-eno-pyranosonic acid which comprises the reaction of 5-acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonic acid with a compound of formula (I)



(I)

where at least one of X^1, X^2, X^3 and X^4 is C-R and the remainder are C-R or N

and each R is the same or different and is H, C_{1-6} alkyl, unsubstituted or substituted phenyl, unsubstituted or substituted phenyl C_{1-4} alkyl or an electron withdrawing group.

2. A method as claimed in claim 1 wherein the compound of formula (I) is pyrazole-1H-carboxamidine or a derivative thereof bearing a C_{1-6} alkyl group on the pyrazole ring.

3. A method as claimed in claim 2 wherein the compound of formula (I) is pyrazole-1H-carboxamidine.

4. A method as claimed in any one of claims 1 to 3 wherein the compound of formula (I) is in the form of an acid addition salt.

5. A method as claimed in any one of claims 1 to 4 wherein the salt is the hydrochloride salt.

6. A method as claimed in any one of claims 1 to 5 wherein the reaction is carried out in an aqueous medium.

7. A method as claimed in claim 6 wherein the aqueous medium is water.

8. A method as claimed in any one of claims 1 to 7 wherein the process is carried out at a temperature of 30-70°C.

9. A method as claimed in claim 8 wherein the temperature is from 50-55°C.

10. A method as claimed in any one of claims 1 to 9 wherein the molar ratio of 5-acetamido-2,3,4,5-tetradeoxy-4-amino-D-glycero-D-galacto-non-2-eno-pyranosonic acid to the compound of formula (I) is in the range of 1:1 to 1:10.

11. A method as claimed in claim 10 wherein the ratio is about 1: 1.8.

12. A method as claimed in any one of claims 1 to 11 wherein the pH is in the range of 6-9.

13. 5-Acetamido-2,3,4,5-tetradeoxy-4-guanidino-D-glycero-D-galacto-non-2-eno-pyranosonic acid whenever prepared by the method of any one of claims 1 to 12.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 93/02575

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C07D309/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LIEBIGS ANNALEN DER CHEMIE vol. 1991, no. 2 , February 1991 , WEINHEIM pages 129 - 134 ERWIN SCHREINER ET. AL. "Article"	1-13
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INTERNATIONAL SEARCH REPORT

mation on patent family members

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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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